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The influence of freezing and duration of storage on *Campylobacter* and indicator bacteria in broiler carcasses

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Abstract

In total, 215 commercially processed broiler carcasses were examined to determine optimum cultural enumeration, the effects of freezing, method of thawing, and duration of frozen storage on levels of *Campylobacter* spp. and fecal coliforms. Enumeration studies compared MPN procedures to direct plating onto selective mCCDA agar and indicated equivalency for quantitation of *Campylobacter* spp. Levels of *Campylobacter* and fecal coliforms were subsequently estimated by direct plating of carcass rinses. Freezing of naturally contaminated carcasses followed by storage at −20 °C for 31, 73, 122 and 220 days showed statistically significant ($P \leqslant 0.05$) reductions in *Campylobacter* counts initially as compared with counts found on fresh product. Among 5 lots of broilers, levels of *Campylobacter* on carcasses were reduced by log mean values ranging from 0.65 to 2.87 after freezing and 31 days of storage. Similar reductions due to freezing were not observed for fecal coliforms counts. The level of *Campylobacter* was reduced by approximately one log immediately after freezing, and remained relatively constant during the 31–220 days of frozen storage. The levels were constant during 7 days of refrigerated storage. After 31 days of frozen storage there was a reduced rate in reduction of counts among broilers thawed at 7 °C as compared to thawing at 22 °C with either cultural method (MPN and mCCDA). These findings warrant consideration of the public health benefits related to freezing contaminated poultry prior to commercial distribution to reduce *Campylobacter* exposure levels associated with contaminated carcasses.

Keywords: *Campylobacter*; Fecal coliforms; Enumeration; Freezing/thawing/chilling; Storage; Poultry

1. Introduction

Campylobacter jejuni is the most frequent cause of foodborne bacterial infection in many developed countries (Altekruse et al., 1999; Friedman et al., 2000). The organism has been isolated from various food sources such as poultry, red meat, and raw milk (Atanassova and Ring, 1999). Epidemiological studies indicate that handling raw poultry or eating undercooked poultry are important risk factors for transmitting campylobacteriosis in many industrialized countries. Additional identified risk factors for humans include consumption of meats other than poultry, drinking untreated surface water, or drinking unpasteurized milk and dairy products (Friedman et al., 2000).

High frequencies and levels of *Campylobacter* spp. in poultry products have been reported among numerous developed countries. Atanassova and Ring reported an incidence of 45.9% contamination in broiler carcasses in Germany (1999). Ono and Yamamoto reported a prevalence of 45.8% in domestic retail poultry in Japan (1999). Madden and co-workers reported a 38% frequency in cut-up chicken portions in Northern Ireland (1998). The Icelandic surveillance program for Campylobacter

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reported an annual flock prevalence of 13.7–19.2% in ceaca samples from processed broiler flocks from 2000 to 2004 (Icelandic Veterinary Services, 2004). Various levels of *Campylobacter* have been reported in poultry products. Berrang et al. (2001) reported concentrations ranging from 2 to 3 log 10 cfu g^{-1} of *Campylobacter* in breast skin, thigh skin and drumstick skin samples and significantly lower concentration in the corresponding meat samples. Whyte et al. (2001) recovered 1.88–3.59 log 10 cfu g^{-1} of *Campylobacter* from neck skins, depending on the makeup of a water-bath in which poultry carcasses were immersed. Stern and Robach (2003) reported the average level of *Campylobacter* as $10^{4.11}$ cfu $carcass^{-1}$ in 1995 being reduced to$10^{3.05}$ cfu $carcass^{-1}$ in 2001 among north Georgia (United States), processed whole broiler rinses. Although the levels were greatly reduced, the frequency remained quite high, with approximately 85% of the carcasses sampled being contaminated.

A positive relationship between the exposure to pathogenic *Campylobacter* cells and the probability of infection has been published (Black et al., 1998). This relationship suggests that a significant reduction of viable *Campylobacter* spp. in commercial poultry products could result in a reduced incidence of *Campylobacter* infections in humans. A number of chemical or physical decontamination methods have been investigated to control the level of *Campylobacter* and other human pathogens on broiler carcasses including ozonation, super-chlorination, organic acids and steam pasteurization (Whyte et al., 2003).

While *Campylobacter* spp. routinely demonstrate survival on broiler carcasses held under refrigeration (Stern, 1995), freezing is known to decrease the viability of some bacteria including *Campylobacter*. The loss of viability has been attributed to ice nucleation and dehydration. Stead and Park (2000) demonstrated that *Campylobacter* encounter oxidative stress during freezing and thawing and that superoxide dismutase plays an important role as a resistance mechanism for cells to overcome this stress. Studies, both in pure broth cultures (Humphrey and Cruickshank, 1985) and on naturally contaminated broiler carcasses (Stern et al., 1985), ground beef livers (Hänninen, 1981) and artificially contaminated beef trimmings (Moorhead, 2002), which were frozen and thawed, showed reductions in levels of *C. jejuni* or *C. coli*. Moore et al. (2002) observed that 94% of fresh poultry samples ($n = 63$) vs. 77% of frozen samples ($n = 44$) indicated the reduction of *Campylobacter* spp. during the poultry freezing process. Lee et al. (1998) demonstrated that *C. jejuni* artificially inoculated onto chicken skin survives freezing under various atmospheric conditions. Their data did indicate a rapid reduction in survival during the first 2 weeks of −20 °C storage.

Human campylobacteriosis reached epidemic proportions in Iceland in 1999 as compared to 1997 (Stern et al., 2003). Levels of domestic origin had risen from less than 20/100,000 to 116/100,000 and the Public Health authorities were under considerable public pressure to reduce the problem. Fresh poultry was identified as the likely source for this upsurge in disease. In 2000, the Icelandic official veterinary authorities decided to employ freezing of poultry products as a mitigation strategy to reduce human exposure to *Campylobacter*. The central purpose of the current study was to quantify the effects of freezing and frozen storage on the numbers of *Campylobacter* and fecal coliforms in naturally contaminated broilers. As part of this study we compared two *Campylobacter* enumeration procedures, and assessed the effect of cold storage on the survival of the organism.

2. Materials and methods

All samples in these studies were obtained from two poultry processing plants in Iceland; following evisceration, processing plant A uses immersion chilling of broiler carcasses and processing plant B uses spray chilling. These represent two of the four poultry processing plants operated in Iceland and produce approximately 90% of the broiler meat consumed in the country.

2.1. Trial 1—Long-term, frozen carcass storage and sampling

A total of 90 moisture-impervious, plastic heat-sealed broiler carcasses were obtained from processing plant A. All the carcasses were from the same flock of broilers, harvested at 5–6 weeks of age, which had tested positive for *Campylobacter* at 4 weeks of age. All samples were gathered within $\frac{1}{2}$ h from the processing line. The weight of the sample carcasses ranged from 900 to 1300 g. Immediately after packaging, ten fresh carcasses were transported to the laboratory, stored at 2–4 °C and analysed within 24 h. The remaining 80 carcasses were frozen at the processing plant and then stored at −20 °C during the trial period. The frozen carcasses were divided into four groups containing 20 carcasses each. After storage times of 31, 73, 122 and 220 days, one group of carcasses was transported to the laboratory for microbiological analysis. At the laboratory, each group was divided into two groups of ten carcasses. One group was thawed at 7 °C for 20 h and the other group at 22 °C for 16 h before analysis.

Similar to the methods as described by Stern and Robach (2003), each individual carcass sample was weighed and aseptically placed into a sterile plastic bag. To each bag, 225 ml of sterile buffered peptone water (BPW; CM509:Oxoid) was added and each carcass vigorously shaken by hand for 60 s. After rinsing, the carcass was aseptically removed and the rinse BPW used for microbiological analysis.

2.2. Trial 2—Freezing and 31-day frozen storage

In trial 2, four additional 5–6-week-old flocks of *Campylobacter* positive broilers (as determined by

sampling at 4 weeks of age) were sampled from processing plant B as described for trial 1. The carcass samples from each flock were collected on 4 separate slaughter days over a period of 16 weeks. A total of 20 moisture-impervious, plastic heat-sealed broiler carcasses were obtained from each flock. These were divided into two groups, 10 fresh carcasses that were transported to the laboratory and analysed within 24 h and the remaining 10 that were held frozen at the processing plant. The frozen carcasses were stored at −20 °C for 31 days and thawed at 7 °C for 20 h before analysis. The weight of the carcasses from these four flocks ranged from 1100 to 1800 g, 1000 to 1300 g, 1100 to 1300 g and 800 to 1600 g, respectively.

Each carcass sample was weighed and rinsed with BPW as described in trial 1.

2.3. Trial 3—Short-term, frozen-storage at −20 °C and cold storage at 3 °C

In this trial 45 plastic-packaged carcasses were obtained from a single flock of known positive broilers from processing plant B. The carcasses were sampled as described in trial 1. From these samples, 30 chilled, fresh carcasses were transported to the laboratory and 10 carcasses were analysed within 24 h from the time of processing. Twenty carcasses were divided into 4 groups of 5 each that were subsequently analysed after 1, 2, 4 and 7 days storage at 3 ± 1 °C. The remaining 15 carcasses were frozen at the processing plant and divided into 3 groups of 5 each that were stored at −20 °C for 1, 6 and 9 days. These carcasses were then thawed at 7 °C for 20 h and analysed. Each carcass was weighed and rinsed in BPW as described in trial 1.

2.4. Microbial analysis

Trial 1. Each sample was quantitatively analysed for *Campylobacter* using two methods; a direct plating technique on a modified cefoperazone charcoal deoxycholate agar (mCCDA) (CM739: Oxoid supplemented with SR155:Oxoid) and MPN technique with Preston broth (CM67:Oxoid supplemented with SR204E:Oxoid and SRO84E:Oxoid and 5% horse blood). The methods are described in detail by Line et al. (2001).

Decimal dilutions of the BPW used to rinse the carcasses were made in saline-peptone water. Volumes of 0.5 and 0.1 ml from the undiluted samples and 0.1 ml of 10^{-1}–10^{-4} dilutions were surface plated on mCCDA plates. Plates that received 0.5 ml were dried for 60 min at 37 °C to obtain separated colonies. Other plates were dried for 30 min at 37 °C. The inoculated plates were incubated at 41.5 °C for 48 h under microaerobic conditions generated using the CampyGen™ *Campylobacter* System (CN35:Oxoid). Following incubation, suspect colonies, preferentially from plates containing 15–150 colonies, were counted and three colonies from each plate were confirmed using colony morphology, Gram stain and biochemical tests including hippurate hydrolysis and indoxylacetate hydrolysis. The results were calculated as cfu of thermophilic *Campylobacter* spp. ml^{-1} of the 225 ml-BPW rinse fluid.

Volumes of 10 and 1 ml from the undiluted samples and 1 ml of 10^{-1}–10^{-4} dilutions were assayed by a three-tube MPN technique into tubes of Preston broth. Tubes containing 90 ml of Preston broth were used for 10 ml sample volumes and tubes with 9 ml of Preston broth for 1 ml sample volumes. After inoculation the tubes were filled to approximately $\frac{3}{4}$ of the tubes volume capacity. The tubes were closed with tight-fitting caps and incubated at 37 °C for 3 h. The inoculated tubes were then removed from the incubator and supplements (SR204E:Oxoid and SRO84E:Oxoid) containing selective antibiotics were added using a micropipette. After an additional 45 h of incubation at 37 °C a loop full was streaked from each tube on mCCDA plate (one plate divided into three parts for every three tubes). After incubation at 41.5 °C for 48 h under microaerobic atmosphere, suspect colonies of *Campylobacter* were picked and confirmed as described above. The MPN of thermophilic *Campylobacter* spp. ml^{-1} of BPW was then calculated from a standard MPN table.

From the same samples, fecal coliforms were analysed with a pour plate method, starting with 1 h preincubation at 37 °C on tryptone soya agar (TSA) (CM 131:Oxoid) followed by overlay of violet red bile lactose agar (VRBLA) (CM 107:Oxoid) and incubation at 44 °C for 22 h (Nordic Committee on Food Analysis (NMKL), 1996). Volumes of 1 ml from the undiluted samples and 1 ml of 10^{-1}–10^{-4} dilutions were pour-plated with TSA agar and VRBLA as above. After incubation typical coliform colonies were counted and 5 colonies of each colonial type were confirmed by inoculation into EC broth (Difco 0314-17) at 44 °C for 24 h to test for gas production. The results were calculated as cfu of fecal coliforms ml^{-1} of the BPW rinse water.

Trial 2. Each sample was analysed quantitatively for *Campylobacter* and fecal coliforms using the same method procedure and calculations as described in trial 1 excluding the MPN method for *Campylobacter*.

Trial 3. Each sample was analysed quantitatively for *Campylobacter* using direct plating technique onto mCCDA and employing the same procedures and calculations as described in Trial 1.

2.5. Statistical analysis

All bacterial counts calculated in the BPW rinse were expressed as bacterial counts 1000 g^{-1} broilers transformed to $\log_{10}$ values for subsequent data analysis. The effect of freezing, and frozen or cold storage on bacterial counts was compared statistically using unpaired Student's *t*-tests with significance defined at the 95% level ($P \leqslant 0.05$).

3. Results

Trial 1—Long-term, frozen carcass storage. Freezing, followed by storage at −20 °C for 31, 73, 122 and 220 days

of naturally contaminated broilers caused statistically significant ($P \leqslant 0.05$) reductions in *Campylobacter* counts as compared with the counts observed in the fresh product (Table 1). The only exception was observed with the MPN results from broilers thawed at 7 °C after 31 days of frozen storage.

After 31 days of storage there was a reduced rate in reduction of counts among broilers thawed at 7 °C as compared to thawing at 22 °C with either cultural method (MPN and mCCDA). With extended storage the reduction seemed to be similar at both thawing temperatures, except after 73 days when, without account, the counts for carcasses thawed at 7 °C were lower then with those thawed at 22 °C ($P \leqslant 0.05$). After 73 days of storage the counts of surviving *Campylobater* seem to have stabilized and there was no significant reduction during the remaining 220 days of storage time. As the results from this long-term trial did not show very clear distinction in reduction rate of *Campylobacter* counts when thawed at 22 °C compared to thawing at 7 °C, it was decided to use only 7 °C in subsequent trials.

The results from the direct plating technique on mCCDA were consistently lower than the results obtained with the MPN technique but without statistical significance ($P \leqslant 0.05$). It was then decided to only use the easier and more efficient direct plating onto mCCDA method for the subsequent trials.

Freezing followed by frozen storage of upto 220 days caused small reduction in the number of fecal coliforms recovered from the carcass rinses, when compared to freshly processed samples, regardless of whether thawed at 7 or 22 °C. Thawing at 7 °C caused slightly greater reductions in counts than did thawing at 22 °C. All results after freezing, except for those samples thawed at after 73 days of storage, yielded statistically insignificant reductions for fecal coliforms as compared to the results observed before freezing. Apart from samples that were thawed at 7 °C following 73 days of frozen storage, no statistically significant reductions for the fecal coliforms were seen as compared to the prestorage fresh samples.

Trial 2—Freezing and 31-day frozen storage. Table 2 provides data demonstrating the statistically significant ($P \leqslant 0.05$) reductions in *Campylobacter* counts among the five broiler flocks tested. These data were derived following freezing and short-term frozen storage of carcasses at −20 °C for 31 days as compared to counts observed before carcass freezing. The mean reduction observed for each broiler flock ranged from 0.65 to 2.87 $\log_{10}$ cfu 1000 g^{-1} broiler.

Levels of fecal coliforms were limitedly reduced following the freeze treatment in all broiler flocks, except for flock #3 where the mean count was increased slightly after frozen storage (Table 2). The mean reduction observed for the other flocks ranged from 0.28 to 1.63 $\log_{10}$ cfu 1000 g^{-1} broiler, being statistically significant ($P \leqslant 0.05$) for flocks #2, #4 and #5. When compared to mean reductions in *Campylobacter* counts, the mean reductions for the fecal coliforms counts were significantly ($P \leqslant 0.05$) less for all flocks except for flock #2 for which the reduction was similar for both groups of micro-organisms.

Figs. 1 and 2 demonstrate the substantial reductions of *Campylobacter* counts compared to levels of fecal coliforms. The combined samples in each category before ($n = 50$) and after ($n = 50$) freezing were grouped into histograms at 1 $\log_{10}$ intervals. For known *Campylobacter* contaminated flocks (Fig. 1), all 50 fresh carcass samples yielded counts between $\log_{10}$ 4–4.99 cfu or higher 1000 g^{-1} broiler. Following freezing and storage, only six samples (12%) had counts $>10^4$ cfu and the remaining 44 samples had $<10^4$ cfu per 1000 g^{-1} broiler. Prior to freezing and storage, levels of fecal coliforms were $>10^4$ cfu per 1000 g^{-1} broiler (Fig. 2), but unlike *Campylobacter,* 48 (96%) of the carcasses still had counts at $>10^4$ cfu after freezing and storage.

Among both the fresh and frozen and stored carcasses, the mean $\log_{10}$ counts were consistently higher for fecal

Table 1
Effects of duration of frozen storage (at −20 °C) on the survival of *Campylobacter* spp. and thermotolerant coliforms in naturally contaminated broiler carcasses

Storage time in freezer (days)	Thawed at 7 °C			Thawed at 22 °C		
	Campylobacter[a,b]		Fecal coliforms[a]	Campylobacter[a,b]		Fecal coliforms[a]
	MPN	mCCD	TSA + VRBA	MPN	mCCD	TSA + VRBA
0[c]	4.74 ± 0.81^{A}	4.66 ± 0.72^{A}	4.83 ± 0.69^{A}	4.74 ± 0.81^{A}	4.66 ± 0.72^{A}	4.83 ± 0.69^{A}
31	4.14 ± 0.82^{A}	4.01 ± 0.78^{B}	4.55 ± 0.50^{A}	3.43 ± 0.67^{B}	3.24 ± 0.63^{B}	4.71 ± 0.45^{A}
73	3.11 ± 0.32^{B}	3.09 ± 0.34^{BC}	4.21 ± 0.48^{B}	3.75 ± 0.77^{B}	3.69 ± 0.87^{B}	4.68 ± 0.58^{A}
122	3.48 ± 0.83^{B}	3.37 ± 0.84^{B}	4.45 ± 0.48^{A}	3.54 ± 0.89^{B}	3.47 ± 0.73^{B}	4.61 ± 0.63^{A}
220	3.81 ± 0.65^{B}	3.32 ± 0.68^{BC}	4.46 ± 0.42^{A}	3.65 ± 0.68^{B}	3.51 ± 0.65^{B}	4.79 ± 0.47^{A}

Note: $n = 10$ broilers samples per analytical group.
± = standard deviation.
Within columns, different letters (A–C) indicate significant ($P \leqslant 0.05$) differences between reported values in those columns.
[a]Results expressed as $\log_{10}$ cfu 1000 g^{-1} broiler.
[b]All confirmed isolates were *C. jejuni*.
[c]Before freezing.

Table 2
Effects of freezing and frozen storage (at −20 °C for 31 days) on *Campylobacter* spp. and fecal coliforms in naturally contaminated broilers thawed at 7 °C

Broiler flock #	*Campylobacter*[a,b]			Fecal coliforms[a]		
	Before freezing	After freezing	Reduction in counts	Before freezing	After freezing	Reduction in counts
1 (ic)	4.66 ± 0.72^{A}	4.01 ± 0.78^{B}	0.65 (77.6%)	4.83 ± 0.69^{A}	4.55 ± 0.50^{A}	0.28 (47.5%)
2 (sc)	4.96 ± 0.49^{A}	3.13 ± 0.46^{B}	1.83 (98.5%)	6.18 ± 0.37^{A}	4.55 ± 0.38^{B}	1.63 (97.7%)
3 (sc)	4.76 ± 0.45^{A}	3.14 ± 0.52^{B}	1.57 (97.3%)	5.26 ± 0.40^{A}	5.65 ± 0.29^{B}	−0.39 (−45.5%)
4 (sc)	5.63 ± 0.85^{A}	3.72 ± 0.26^{B}	1.91 (98.8%)	6.05 ± 0.39^{A}	5.48 ± 0.38^{B}	0.57 (73.1%)
5 (sc)	6.07 ± 0.69^{A}	3.20 ± 0.31^{B}	2.87 (99.9%)	6.43 ± 0.53^{A}	5.65 ± 0.20^{B}	0.78 (83.4%)

Note: $n = 10$ broilers samples per analytical group.
$\pm$ = standard deviation.
ic: immersion chilling.sc: spray chilling.
For each flock, within Campylobacter or fecal coliform columns, different letters (A or B) indicate significant ($P \leqslant 0.05$) differences between results before (A) and after (B) freezing.
[a]Results expressed as $\log_{10}$ cfu per 1000 g broiler.
[b]All confirmed isolates were *C. jejuni*.

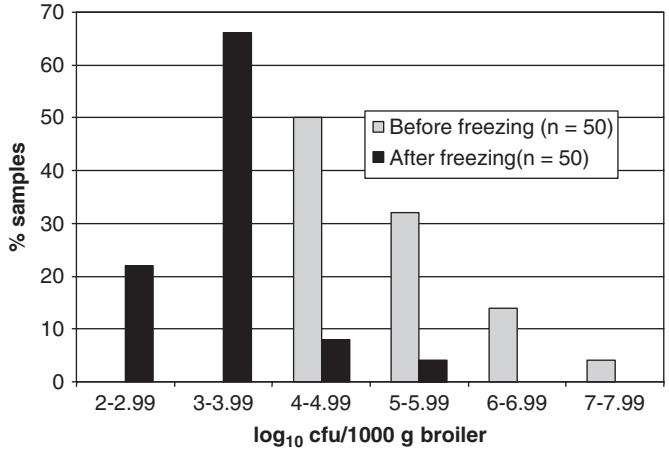

Fig. 1. Distribution of *Campylobacter* counts ($\log_{10}$ cfu 1000 g^{-1} broiler) in broiler carcasses before and after freezing and frozen storage at −20 °C for 31 days.

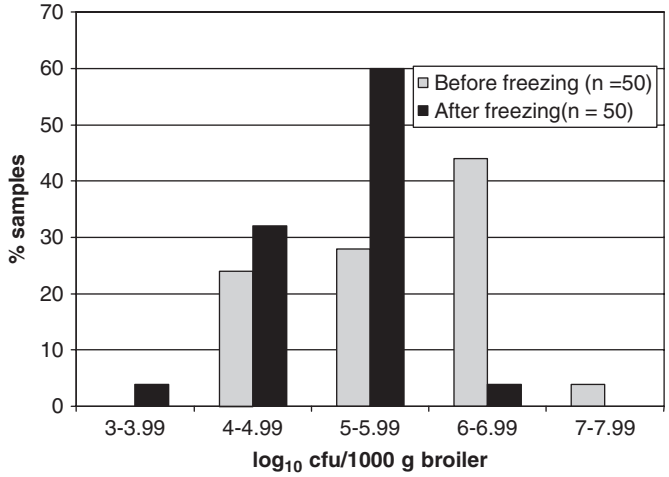

Fig. 2. Distribution of fecal coliform counts ($\log_{10}$ cfu 1000 g^{-1} broiler) in broilers before and after freezing and frozen storage at −20 °C for 31 days.

coliforms compared to levels of *Campylobacter* in the flocks sampled (Table 2). These relative numbers indicate that the *Campylobacter* population is more sensitive to freezing than the fecal coliforms originating within the broiler intestinal tract. The mean $\log_{10}$ cfu ratio of fecal coliforms relative to *Campylobacter* was 1.1 (5.75/5.22) for the fresh product and for the frozen and stored product the ratio was 1.5 (5.18/3.45). This suggested that fecal coliforms survive frozen and storage conditions in broiler carcasses better than *Campylobacter*.

Trial 3—*Short-term, frozen storage at* −20 °*C and cold storage at* 3 °*C* (*Table* 3). There was greater than 1 $\log_{10}$ reduction in *Campylobacter* counts after 9 days of storage at −20 °C when compared to the counts observed in the fresh broiler samples. Already after only 1 day in the freezer the reduction was significant ($P \leqslant 0.05$) and was approximately 1 $\log_{10}$. The counts of C*ampylobacter* in broiler carcasses stored in the refrigerator decreased insignificantly from the initial counts throughout the storage period.

4. Discussion

Table 1 provides data comparing levels of *Campylobacter* spp. estimated by employing a most probable number (MPN) method to a direct plating measurement. The MPN method requires approximately 1 additional day for analysis than does the direct plating approach and does not provide any statistical advantage in estimations of numbers. This procedure saves time and might be valuable if product was stored prior to release. An estimated 10-fold increase in numbers of samples can be processed by a laboratory using direct plating as compared to using a MPN approach. Fewer mathematical mistakes are likely with the direct plating method when decimal dilutions are correctly accounted in the calculations. It is also recognized that the MPN approach encompasses a 95% confidence interval leading to a large variation in the estimated

numbers as compared with direct plating. With larger numbers of samples made possible through direct plating, an improved estimation of the level of contamination within a process lot can be provided. Also, the cost of direct plating is less than the cost for MPN. Direct plating on mCCDA agar, as used in this study, appears to be a suitable method for direct, simple, and accurate enumeration of *Campylobacter* in poultry rinses. Line et al. (2001) has also demonstrated that direct plating onto Campy-Cefex medium provided similar estimations of *Campylobacter* levels as did use of MPN techniques.

The initial load of *Campylobacter* on contaminated fresh broiler carcasses ranged from $10^{4.66-6.07}$ cfu $1000\,g^{-1}$ broiler (Table 2). The levels of *Campylobacter* on fresh broiler carcasses from the present study provide comparable values published in the literature. In general, the literature does not report the weight of the carcass samples, while we chose to estimate levels per 1000 g of carcass. Birds in Iceland are typically processed around 5–6-weeks of age and "dress-out" around this weight. Smeltzer (1981) found processed carcasses to be contaminated with up to 10^5 cfu per broiler carcass in Australia. Hood et al. (1988) found levels of *Campylobacter* in United Kingdom broilers of $>10^6$ cfu per carcass. Stern and Robach (2003) used similar direct plating procedures as employed in the present study to estimate the levels of *Campylobacter* on freshly processed carcasses. They reported that north Georgia flock mean levels (50 carcasses sampled per flock) ranged from $10^{3.01-4.59}$ cfu per carcass. In that study, the highest level reported per individual carcass was estimated to be $10^{6.50}$ cfu.

A decrease in *Campylobacter* counts was most rapid immediately after the carcasses were frozen (Tables 1 and 3). This observation is consistent with those originally presented by Hänninen (1981) and corroborated by Stern et al. (1985), and Humphrey and Cruickshank (1985), who described the fragility of the organism relative to the freezing process. Further loss of viability was limited during the first week of frozen storage, with an additional half-log reduction observed after a month and another additional ~half-log reduction observed after 7 months of storage. The reduced levels of *Campylobacter* spp. in carcass rinses after the initial freezing reductions may be accounted by increased ice crystal formation during the repetitive mechanical freezing process or, by desiccation of the product over the storage period.

The reduction in levels of *Campylobacter* after freezing of contaminated chicken carcasses reduces subsequent exposure during handling and likely reduces the risk of illness. Black et al. (1998) indicated that there was a relationship between challenge dose and human disease. The organism will not proliferate below temperatures of 32 °C, which precludes any increase in numbers of *Campylobacter* under normal poultry storage conditions. Stern et al. (2003) reported that in 1999, Iceland had a rate of approximately 116 cases per 100,000 people due to domestic origin. The following year, when the Iceland poultry industry began to voluntarily freeze carcasses of known infected flocks, the human rate for disease dropped to 33 per 100,000. Although this voluntary procedure did not likely account for the entire reduction in human disease, it certainly did contribute. As shown in Fig. 1, there would be greater than 10-fold reduction in human exposure after freezing when compared to the chilled fresh carcasses. We submit that if all human processing errors are equal, such a decrease in exposure on the carcasses will reduce the risk of human infections. We would also note that there was a major public awareness campaign that was begun in 2000 to educate consumers to employ home hygienic measures to prepare poultry safely and apply adequate time-temperature measures known to kill the organism.

To reduce the disease burden of human campylobacteriosis attributed to poultry, it may be feasible to implement multiple hurdles along the farm to table continuum of the product. Freezing of known positive poultry carcasses appears to have provided benefit for reducing *Campylobacter*. This hurdle was implemented in Iceland in 2000 and Norway adapted the same strategy in 2001. Additional hurdles might include additional biosecurity measures in

Table 3
Effects of short-term, frozen storage at −20 °C and cold storage at 3 °C on the survival of *Campylobacter* spp. in naturally contaminated broilers

Time in freezer (days)	*Campylobacter*[a,b]	Time in cold storage (days)	*Campylobacter*[a,b]
0[c]	5.67 ± 0.81^{A}	0	5.67 ± 0.81^{A}
1	4.76 ± 0.43^{B}	1	5.47 ± 0.29^{A}
6	4.28 ± 0.13^{BC}	2	5.06 ± 0.44^{A}
9	4.42 ± 0.27^{B}	4	4.99 ± 0.67^{A}
		7	5.26 ± 1.14^{A}

Note: $n = 10$ broilers at day 0 and n = 5 at other days.
$\pm$ = standard deviation.
Within columns, different letters (A–C) indicate significant ($P\leqslant0.05$) differences between results before (A) and after freezing (B, C). C is significantly ($P\leqslant0.05$) different from B.
[a]Results expressed as $\log_{10}$ cfu per 1000 g broiler.
[b]All confirmed isolates were *C. jejuni*.
[c]Before freezing.

primary production, the addition of lactic acid treatments during immersion chilling, and improved knowledge and education among food handlers in the home as well as those employed in food service.

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References

Altekruse, S.F., Stern, N.J., Fields, P.I., Swerdlow, D.L., 1999. *Campylobacter jejuni*—an emerging foodborne pathogen. Emerg. Infect. Dis. 5, 28–35.

Atanassova, V., Ring, C., 1999. Prevalence of *Campylobacter* spp. in poultry and poultry meat in Germany. Int. J. Food Microbiol. 51, 187–190.

Berrang, M., Buhr, R.J., Ladely, S.R., 2001. Presence and level of *Campylobacter,* coliforms, *Escherichia coli*, and total aerobic bacteria recovered from broiler parts with and without skin. J. Food Prot. 64, 184–188.

Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P., Blaser, M.J., 1998. *Campylobacter jejuni* infection in humans. J. Infect. Dis. 157, 472–479.

Friedman, C.R., Neimann, J., Wegener, H.C., Tauxe, R.V., 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin, X., Blaser, M.J. (Eds.), Campylobacter I. American Society for Microbiology, Washington, DC, pp. 121–138.

Hänninen, M.-L., 1981. Survival of *Campylobacter jejuni/coli* in ground refrigerated and in ground frozen beef liver and in frozen broiler carcasses. Acta Vet. Scand. 22, 566–577.

Hood, A.M., Pearson, A.D., Shahamat, M., 1988. The extent of surface contamination of retailed chickens with *Campylobacter jejuni* serogroups. Epi. Infect. 100, 17–25.

Humphrey, T.J., Cruickshank, J.G., 1985. Antibiotic and deoxycholate resistance in *Campylobacter jejuni* following freezing and heating. J. Appl. Bacteriol. 59, 65–71.

Icelandic Veterinary Services, 2004. In Annual Report for 2004, pp. 25–26. http://www.yfirdyralaeknir.is/pdf_skjol/arsskyrslaEYD2004.pdf.

Lee, A., Smith, S.C., Coloe, P.J., 1998. Survival and growth of *Campylobacter jejuni* after artificial inoculation onto chicken skin as a function of temperature and packaging conditions. J. Food Prot. 61, 1609–1614.

Line, J.E., Stern, N.J., Lattuada, C.P., Benson, S.T., 2001. Comparison of methods for the recovery and enumeration of *Campylobacter* from freshly processed broilers. J. Food Prot. 64, 982–986.

Madden, R.H., Moran, L., Scates, P., 1998. Frequency of occurrence of *Campylobacter* spp. in red meats and poultry in Northern Ireland and their subsequent subtyping using polymerase chain reaction-restriction fragment length polymorphism and the random amplified polymorphic DNA method. J. Appl. Microbiol. 84, 703–708.

Moore, J.E., Wilson, T.S., Wareing, D.R.A., Humphrey, T.J., Murphy, P.G., 2002. Prevalence of thermophilic *Campylobacter* spp. in ready-to-eat foods and raw poultry in Northern Ireland. J. Food Prot. 65, 1326–1328.

Moorhead, S.M., 2002. Survival of *Campylobacter jejuni* on beef trimmings during freezing and frozen storage. Lett. Appl. Microbiol. 34, 72–76.

Nordic Committee on Food Analysis (NMKL), 1996. Thermotolerant Coliform Bacteria. Enumeration in Foods. No. 125. 3rd ed.

Ono, K., Yamamoto, K., 1999. Contamination of meat with *Campylobacter* jejuni in Saitama, Japan. Int. J. Food Microbiol. 47, 211–219.

Smeltzer, T.I., 1981. Isolation of *Campylobacter jejuni* from poultry carcasses. Aust. Vet. J. 57, 511–512.

Stead, D., Park, S.F., 2000. Roles of Fe superoxide dismutase and catalase in resistance of *Campylobacter coli* to freeze-thaw stress. Appl. Environ. Microbiol. 66, 3110–3112.

Stern, N.J., 1995. Influence of season and refrigerated storage on *Campylobacter* spp. contamination of broiler carcasses. J. Appl. Poult. Res. 4, 235–238.

Stern, N.J., Robach, M.C., 2003. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. J. Food Prot. 66, 1557–1563.

Stern, N.J., Rothenberg, P.J., Stone, J.M., 1985. Enumeration and reduction of *Campylobacter jejuni* in poultry and red meats. J. Food Prot. 48, 606–610.

Stern, N.J., Hiett, K.L., Alfredsson, G.A., Kristinsson, K.G., Reiersen, J., Hardardottir, H., Briem, H., Gunnersson, E., Georgsson, F., Lowman, R., Berndtson, E., Lammerding, A.M., Paoli, G.M., Musgrove, M.T., 2003. *Campylobacter* spp. in Icelandic poultry operations and human disease. Epi. Infect. 130, 23–32.

Whyte, P., Collins, J.D., McGill, K., Monahan, C., O'Mahony, H., 2001. Quantitative investigation of the effects of chemical decontamination procedures on the microbiological status of broiler carcasses during processing. J. Food Prot. 64, 179–183.

Whyte, P., McGill, K., Collins, J.D., 2003. An assessment of steam pasteurization and hot water immersion treatments for the microbiological decontamination of broiler carcasses. Food Microbiol. 20, 111–117.